BCN-Azide Ligation

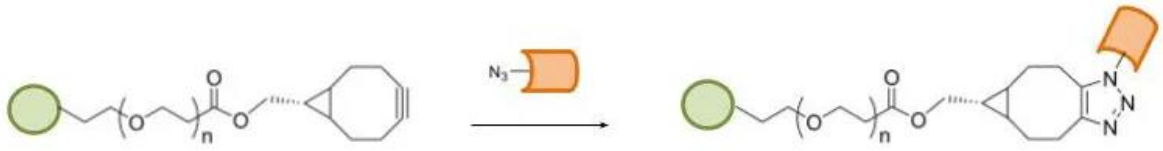

Fig 1. Schematic representation of a BCN-azide ligation reaction

Condition1: EtOH/ H_2O (3:2), room temperature

Condition2: DMSO/H_2O, room temperature

BCN reagent is a class of click chemistry labeling reagents. BCN group can exclusively react with azide-tagged molecules or biomolecules to form a stable triazole. The click chemistry is also known as strain promoted alkyne-azide cycloaddition (SPAAC), BCN reagent has become widely used in bioconjugation, labeling and chemical biology.

BCN click chemistry can be run in aqueous buffer or in organic solvents depending on the property of the substrate molecules. Reagents with PEG arm will increases the compound's hydrophilicity.

This method requires to activate the biomolecule #1 with BCN reagent, and the biomolecule #2 with azide, then to mixing the two activated biomolecules to form a conjugate.

Features

- Biocompatibility – no cytotoxic Copper catalyst required – Nice of in-vivo applications.
- Mild conditions – conjugation in aqueous buffered media and at low temperature
- Stability – BCN and azide moieties are long term stable
- Efficiency – formation of a stable triazole in quantitative yield
- Specificity and Bioorthogonality azide react only with BCN in the presence of -NH2, --COOH and other protein functional group;

Note: Thiol will react BCN, and β-mcercaptoethanol (β-ME) will suppress this thiol-BCN addition.

Example protocol: (Antibody-oligo conjugation)

Antibody activation

- Mix antibody with 20-30-fold molar excess of BCN NHS ester dissolved in DMSO (10 mM solution). DMSO content in the final mixture should be around 20%, antibody concentration in the reaction mixture around 1 mg/mL.
- Incubate at room temperature for 60 minutes.
- Add Tris (10 uL, 100mM in water) to the reaction to quench the unreacted DBCO-NHS ester.
- Incubate for 15 minutes.
- Remove the unreacted BCN-NHS ester using spin desalting column.

Note: BCN-functionalized antibody can be stored at -20°C for up to months.

Conjugation

- Mix BCN-functionalized antibody or another biomolecule with 2-4x molar excess of azide modified oligonucleotide or azide functionalized dye.
- Incubated overnight at room temperature.
- Validate your final conjugate using SDS gel electrophoresis
- Remove the unreacted oligonucleotide or dye using liquid chromatography (reverse phase HPLC, ion exchange HPLC, or both).